

# European Journal of Immunology

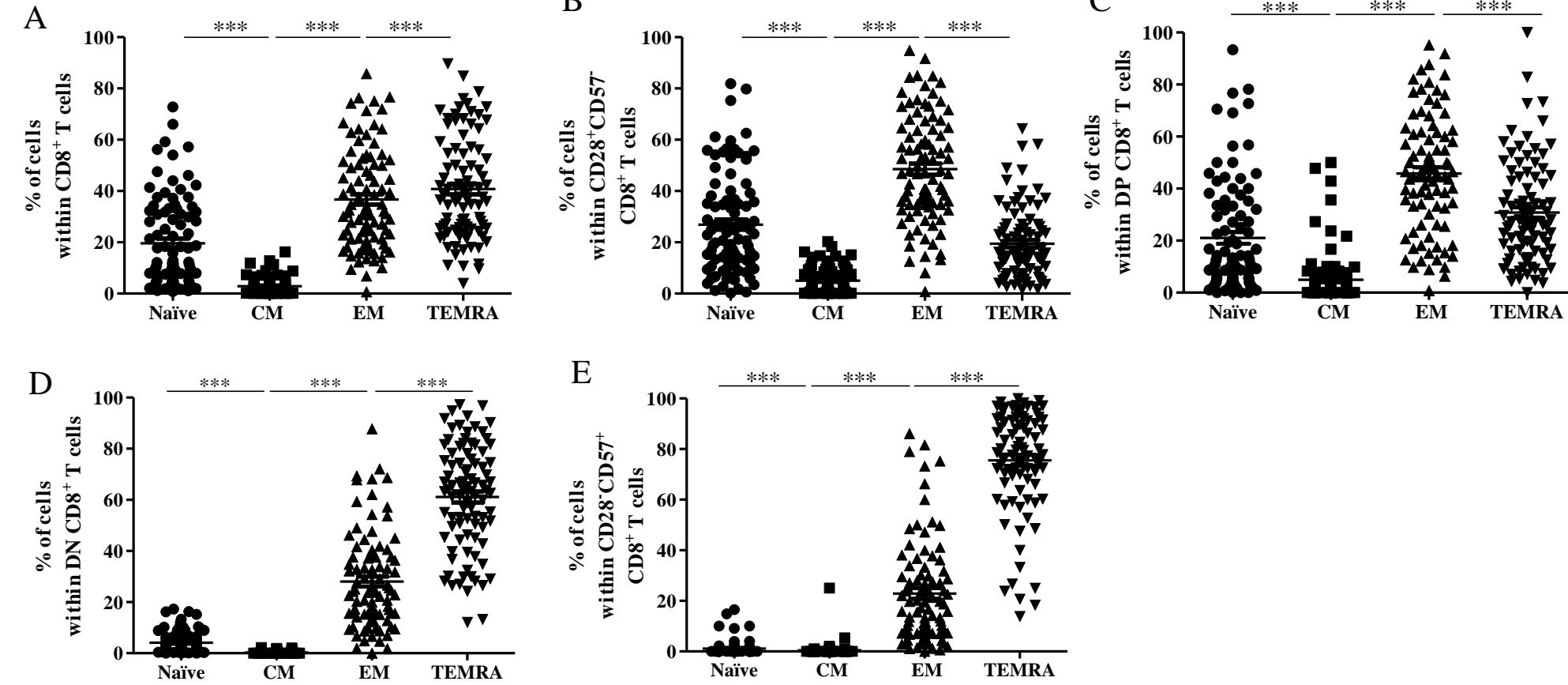
## Supporting Information

for

**DOI 10.1002/eji.201948362**

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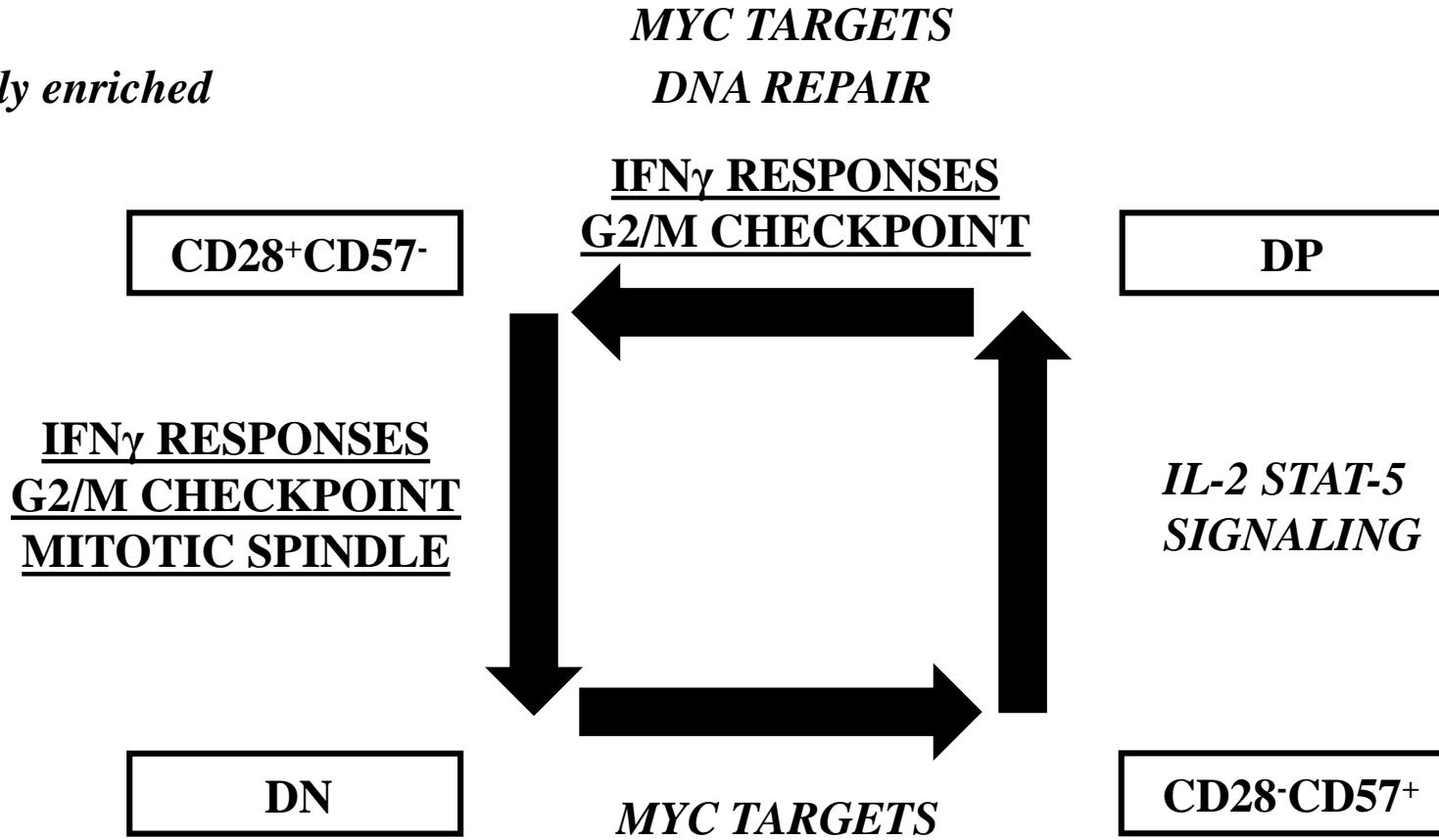
**CD28 and CD57 define four populations with distinct phenotypic properties  
within human CD8<sup>+</sup> T cells**



**Suppl.Fig. 1.** Distribution of naïve, CM, EM and TEMRA. Percentages of naïve, CM, EM and TEMRA within CD8<sup>+</sup> T cells (A), CD28<sup>+</sup>CD57<sup>-</sup> (B), DP (C), DN (D) and CD28<sup>-</sup>CD57<sup>+</sup> (E) CD8<sup>+</sup> T cells. One way ANOVA, Bonferroni *post hoc* test. N=91 in each group. \*\*\*p<0.001.

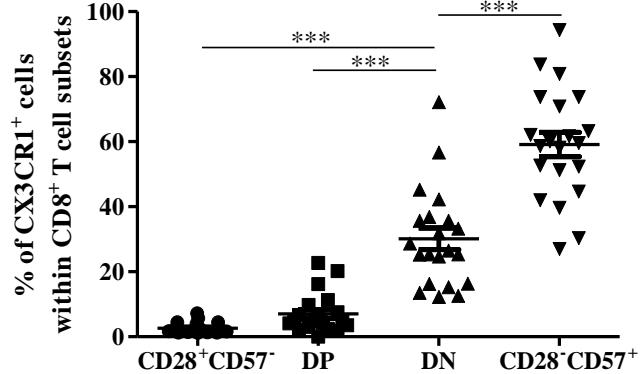
## Pathways positively enriched

## *Pathways negatively enriched*

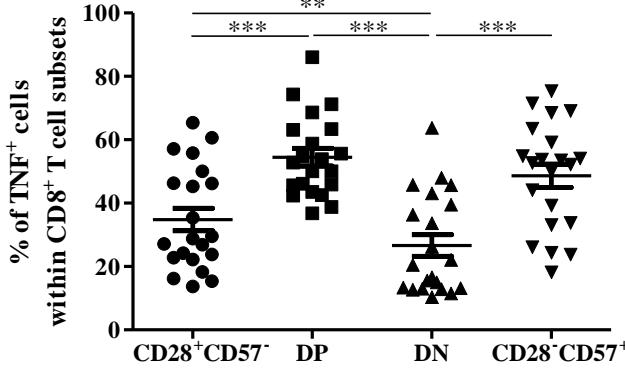


**Suppl.Fig. 2.** Pathways significantly enriched in the comparisons between CD28<sup>+</sup>CD57<sup>-</sup>, DP, DN and CD28<sup>-</sup>CD57<sup>+</sup>CD8<sup>+</sup> T cells after GSEA analysis. Positively and negative enriched pathways are reported in red and in blue respectively. p<0.05, FDR<0.25.

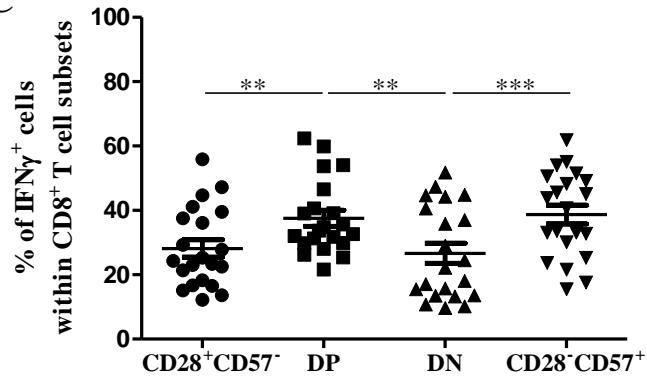
A



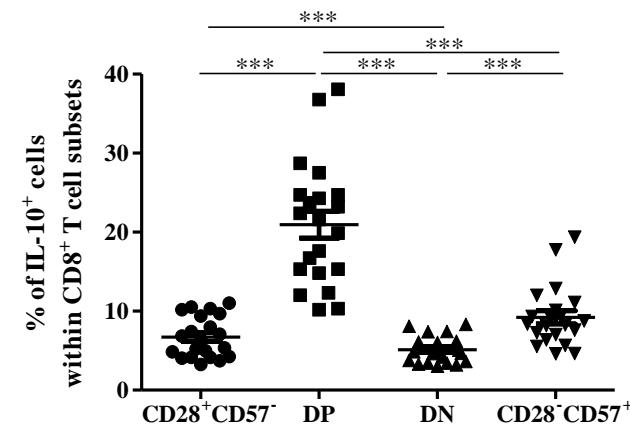
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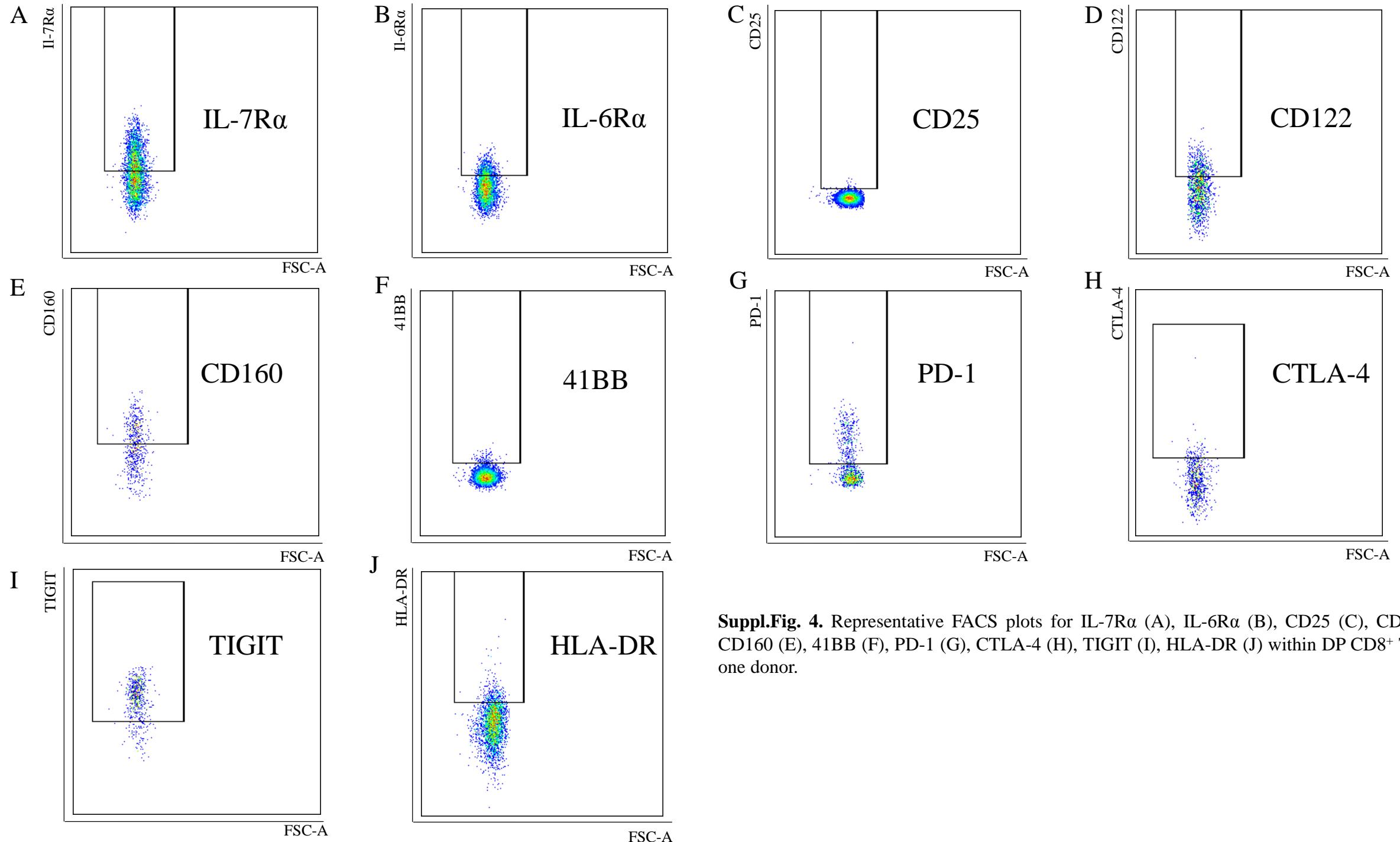
C



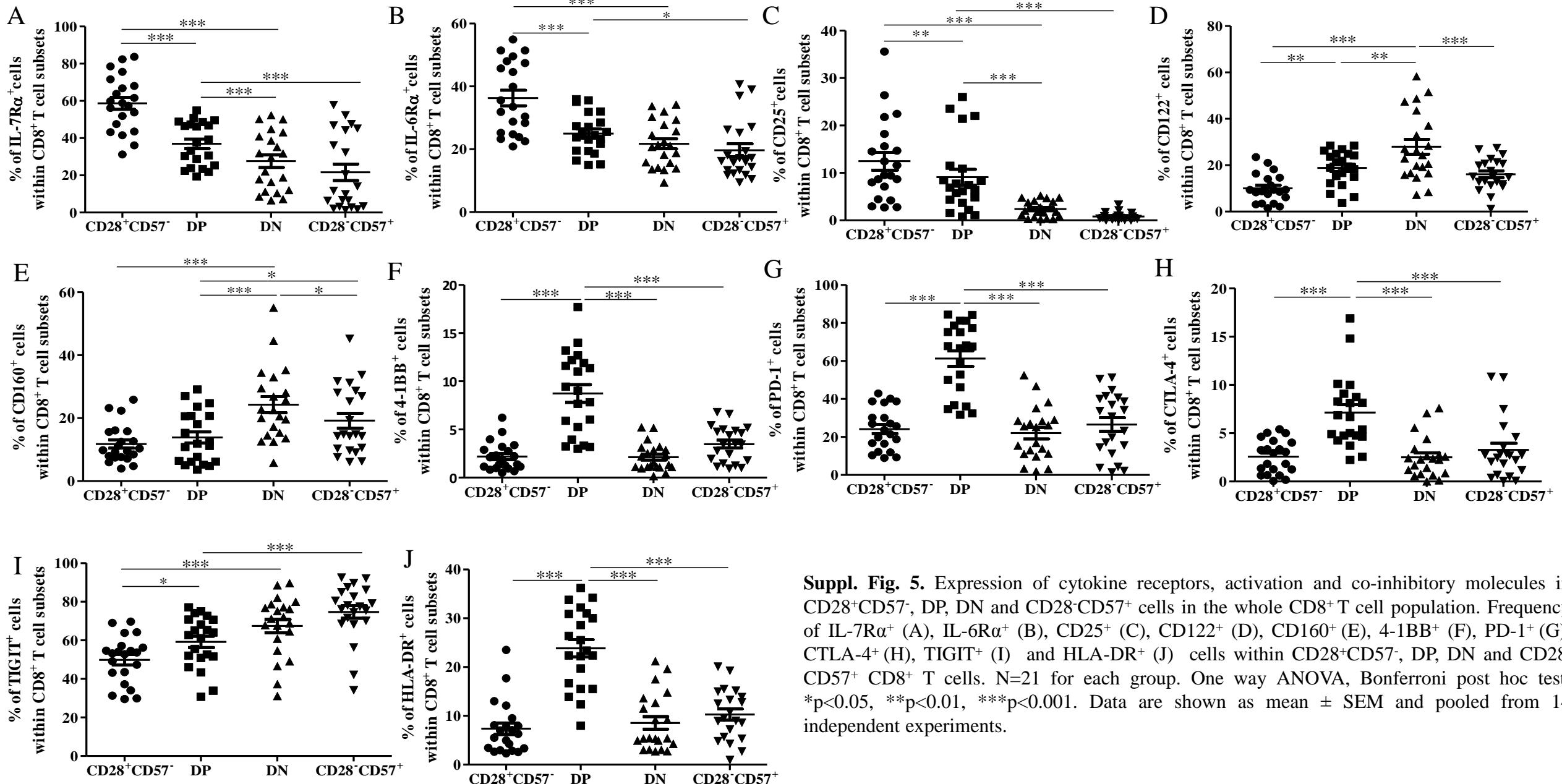
D



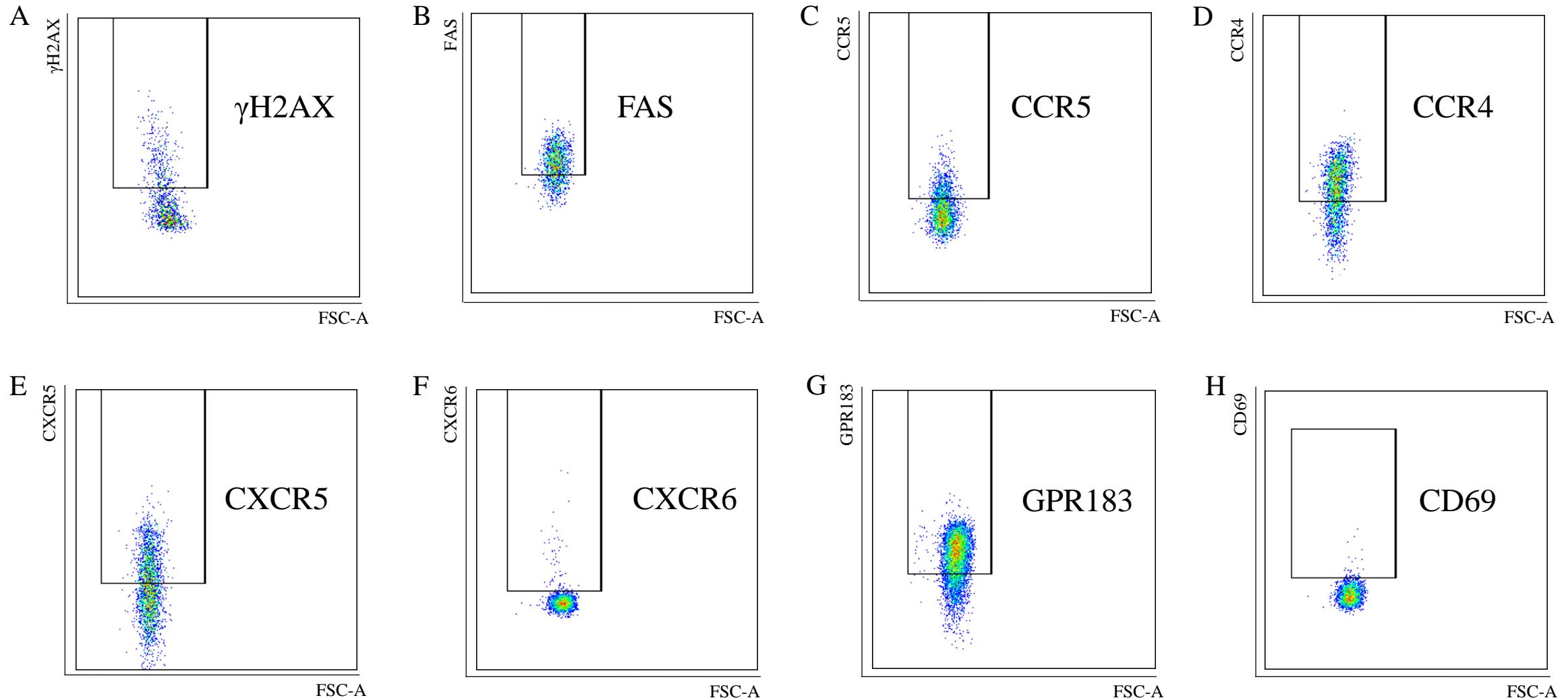
**Suppl.Fig. 3.** Cytotoxic and effector molecules in CD28<sup>+</sup>CD57<sup>-</sup>, DP, DN and CD28<sup>-</sup>CD57<sup>+</sup> cells in the whole CD8<sup>+</sup> T cell population. After gating on CD3<sup>+</sup>CD8<sup>+</sup> T cells within lymphocytes, CD28<sup>+</sup>CD57<sup>-</sup>, DP, DN and CD28<sup>-</sup>CD57<sup>+</sup> subsets were defined. Frequency of CX3CR1<sup>+</sup> (A), TNF<sup>+</sup> (B), IFN $\gamma$ <sup>+</sup> (C), IL-10<sup>+</sup> (D) cells within CD28<sup>+</sup>CD57<sup>-</sup>, DP, DN and CD28<sup>-</sup>CD57<sup>+</sup> CD8<sup>+</sup> T cells. N=21 for each graph. Data are shown as mean  $\pm$  SEM and pooled from 14 independent experiments. One way ANOVA, Bonferroni *post hoc* test. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.



**Suppl.Fig. 4.** Representative FACS plots for IL-7R $\alpha$  (A), IL-6R $\alpha$  (B), CD25 (C), CD122 (D), CD160 (E), 41BB (F), PD-1 (G), CTLA-4 (H), TIGIT (I), HLA-DR (J) within DP CD8<sup>+</sup> T cells in one donor.



**Suppl. Fig. 5.** Expression of cytokine receptors, activation and co-inhibitory molecules in CD28 $^+$ CD57 $^-$ , DP, DN and CD28 $^-$ CD57 $^+$  cells in the whole CD8 $^+$  T cell population. Frequency of IL-7R $\alpha^+$  (A), IL-6R $\alpha^+$  (B), CD25 $^+$  (C), CD122 $^+$  (D), CD160 $^+$  (E), 4-1BB $^+$  (F), PD-1 $^+$  (G), CTLA-4 $^+$  (H), TIGIT $^+$  (I) and HLA-DR $^+$  (J) cells within CD28 $^+$ CD57 $^-$ , DP, DN and CD28 $^-$ CD57 $^+$  CD8 $^+$  T cells. N=21 for each group. One way ANOVA, Bonferroni post hoc test. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001. Data are shown as mean  $\pm$  SEM and pooled from 14 independent experiments.



**Suppl.Fig. 6.** Representative FACS plots for  $\gamma$ H2AX (A), FAS (B), CCR5 (C), CCR4 (D), CXCR5 (E), CXCR6 (F), GPR183 (G) and CD69 (H) within DP CD8<sup>+</sup> T cells in one donor.

Sorted subsets	Timepoints	Subsets (stained after incubation)			
		CD28 <sup>+</sup> CD57 <sup>-</sup>	DP	DN	CD28 <sup>-</sup> CD57 <sup>+</sup>
<b>CD28<sup>+</sup> CD57<sup>-</sup></b>	4h	90.16 ± 2.5	5.66 ± 3.6	3.83 ± 2.1	0.34 ± 0.2
	12h	87.40 ± 2.4	6.57 ± 3.7	5.52 ± 2.7	0.50 ± 0.2
	24h	86.92 ± 2.6	6.84 ± 3.9	5.57 ± 2.9	0.66 ± 0.1
<b>DP</b>	4h	2.45 ± 1.2	91.93 ± 3.5	0.24 ± 0.2	5.69 ± 3.0
	12h	2.56 ± 0.9	90.98 ± 3.2	0.29 ± 0.08	6.15 ± 2.9
	24h	2.89 ± 0.6	90.16 ± 3.1	0.27 ± 0.09	6.66 ± 2.8
<b>DN</b>	4h	1.42 ± 0.3	0.84 ± 0.4	70.56 ± 3.1	27.20 ± 3.1
	12h	1.36 ± 0.2	0.75 ± 0.3	66.33 ± 2.8	31.55 ± 2.8
	24h	1.24 ± 0.1	0.77 ± 0.3	63.33 ± 3.7	34.65 ± 3.9
<b>CD28<sup>+</sup> CD57<sup>-</sup></b>	4h	0.05 ± 0.03	4.61 ± 1.9	0.04 ± 0.03	95.33 ± 1.9
	12h	0.03 ± 0.01	3.44 ± 0.7	0.07 ± 0.01	96.52 ± 0.7
	24h	0.11 ± 0.09	3.53 ± 0.6	0.06 ± 0.04	96.32 ± 0.5

**Suppl. Table 1. Differentiation potential of sorted CD28<sup>+</sup>CD57<sup>-</sup>, DP, DN and CD28<sup>-</sup>CD57<sup>+</sup> CD8<sup>+</sup> T cells incubated for 4h, 12h and 24h.** Sorted CD28<sup>+</sup>CD57<sup>-</sup>, DP, DN and CD28<sup>-</sup>CD57<sup>+</sup> CD8<sup>+</sup> T cells were incubated in the presence of 1µg/ml α-CD3 and 10ng/ml IL-2 for 4h, 12h or 24h and stained with anti-CD3, anti-CD8, anti-CD57 and anti-CD28 Abs. For each condition, frequency of CD28<sup>+</sup>CD57<sup>-</sup>, DP, DN and CD28<sup>-</sup>CD57<sup>+</sup> CD8<sup>+</sup> T cells is shown as mean of 3 samples ± SEM pooled from 3 independent experiments.